

**The effect of aposematism and food calls on the feeding  
behaviour of domestic chicks**

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# 1. Forord

Master graden er ferdig, og studieforløpet nærmer seg slutten. Det har vært en spennende, krevende og ikke minst lærerik tid her på blindern, som jeg ikke ville vært foruten. I den forbindelse er det flere personer jeg ønsker å rette en spesiell takk til.

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## 2. Abstract

Aposematism is a defence system used by toxic animals where the animals use warning colouration, often combined with warning gestures, to fend off predators. The most probable scenario is that the toxicity was developed prior to the warning colouration. Compared to other survival strategies, aposematism do not rely on the prey's capability to hide, run away from or fight off the predator, but rather to be seen and avoided.

Chicks will be influenced by the colour of the food, and how it tastes, to assess if the food is interesting and eatable or not. The hen will use sound to attract chicks to the food source, but will this overrule the aposematic colouration of prey?

My hypothesis is that the chicks will eat most brown prey, with eating fewer bad tasting, and even fewer aposematic bad tasting. Further on my hypothesis is that the chicks that will be given sound during eating will eat more prey, compared to chickens not given sound during eating.

In the experiment 96 domestic chicks, divided into 8 groups (sound or no sound, and bad tasting or neutral prey) were used. The chicks were trained and then tested in 4 days. In the first 12 trials (learning) green and yellow prey tasted bad, and brown tasted good. Green was bad tasting neutral, yellow bad tasting aposematic. In the last 3 trials (extinction learning) green, yellow and brown tasted good.

Yellow prey is eaten more often than green prey during the learning tests, but chicks given yellow prey also show a higher amount of learning from day 1 to day 3, compared to chicks given green prey, although not always confirmed statistically. Sound did have an impact on the chicks attack rate, but it did not follow a clear pattern, and it too also shows no significant difference between different sound groups. In the extinction tests, chicks given green prey shows a higher degree of extinction learning, even though not statistically confirmed, as too with sound.

### **3. Introduction**

#### **3.1 What is aposematism?**

An aposematic signal is defined by Henderson's dictionary of biological terms (2000) as "warning colouration or markings which signal to a predator that an organism is toxic, dangerous or distasteful". So aposematism is a defence system used by potential prey to avoid being eaten, by showing its non-profitability. This is done by manipulating the predator, by sending signals via the use of distinctive colour, odour or behaviour, thus advertising the prey's unprofitability (Mappes, 2005). This would in turn make the predator change their behaviour, and go hunt for some more palatable prey. A problem for the prey would be that there might be predators that do not respond to the aposematic signals, such as if the toxins have no effect on the predator, or if the prey relies on visual stimuli for advertising their unprofitability, while the predator is a non-visual hunter.

#### **3.2 The evolution of aposematism**

Stille and Tullberg (1999) states that it is most likely that the conspicuous traits must have evolved after the animals evolved the toxins it uses as a defence. The reason for this is simply because it would have been devastating for a non-toxic animal to develop phenotypic traits that would make it much easier for a predator to detect it, without having the toxins to back it up (Stille and Tullberg 1999). This seems quite logical, since advertising your whereabouts, would mean that any potential predator would find you more easily, increasing the predation pressure, which in turn could lead to the extinction of this species. Also, a change in the phenotype making an animal more conspicuous would make them more prone for attack by naïve predator initially (Lindstrøm, et.al 1999). The reason for this could be that the weak signals (colouration, etc) did not suffer from an especially high predation rate, but rather that the predator did not learn to separate the unpalatable from the palatable prey initially (Lindstrøm et.al 1999), making the discrimination a learning process. Animals have a tendency to generalise a stimuli, and when the animal generalise in a stimulus dimension after discrimination learning a peak shift often appears (Stille and Tullberg 1999). This means that

after the predator have encountered a stimuli, for instance a red coloured unpalatable beetle, the predator will in the future see all potential prey with red colour as unpalatable, and when encountering prey with even stronger colouration, the predator will avoid this prey even at a higher degree, than the initial prey (Dawkins and Guilford, 1993). Therefore a conspicuous animal will not be attacked and eaten by a predator that has encountered a similar pattern before in an unpalatable prey.

It has been the common assumption that the evolution of aposematic prey happened with a sudden change in morphology, where unpalatable cryptic prey evolved conspicuous traits (Lindstrøm et,al 1999). Essential in this theory is that when a naïve predator encounters a group of prey, it is likely to leave some of the unpalatable prey behind, but any of the prey is likely to be eaten (Endeler, 1988). The alternative to this theory is the gradual change, basically where the cryptic animals gradually evolve a conspicuous trait, which may allow for evolution of aposematism even in solitary prey (Lindstrøm et,al 1999).

### **3.3 Multimodal warning displays**

Multimodal warning displays is something that is often found in animals, such as combinations of odour, rattles, clicks or buzzes, frothing from spiracles, the shaking of brightly coloured wings (Rowe and Guilford, 1999) that aposematic animals produce when attacked. The colouration might be an honest signal of the animals toxicity (Blount *et al*, 2008), with the exception of mimicry. Why the prey animal would produce a certain smell or sound is something that is still debated, both in the way of why they produce them and more importantly how they use them. When faced with a potential predator, it is important for the prey to be able to fend it off, with as little injury as possible, and also to be remembered by the predator as an unprofitable prey. To do so, the prey using aposematism as their defence system, a bright colouration is important for a visual predator, as it will facilitate the learning and maintenance of an avoidance response (Alatalo and Mapps, 1996). Other modalities such as producing a sound or postures will enhance this learning and maintenance response (Rowe, 2002; Gamberale-Stille, 2000).

### **3.4 Chicks food search and the effect of auditory and visual signals**

Chicks (*Gallus gallus domesticus*) have a tendency to eat little food in their first day after hatching, because they tend to do more exploratory pecking of their surroundings (Rogers, 1995). The sight of the chicks is not optimal the first day after hatching (Barnard, 2004) and thereby limiting their pecking and eating. So in these first days, the hen will assist the chicks in finding the food, by vocalization and visual displays (Clarke and Jones, 2001; Kent 1987). Studies have shown that the feeding behaviour of the chickens will be influenced by the hens, via both calls and pecking (Clarke and Jones, 2001). Sound is stated as an important factor of attracting the chickens toward a food source (Collias and Collias, 1956; Horn 2004) and especially if the chickens had heard that sound prior to hatching (Brown\_Grier et.al, 1967). Once the chickens have hatched, factors like frequency, intensity, duration and rate are important to determine the efficiency of the vocalization (Fisher, 1972). Meaning that if the chickens were exposed to hen sounds prior to hatching, something that is highly likely under natural circumstances, the chickens will more easily be affected by hen sounds in their food search, compared to other sounds. Woodcock and Latour (2004) found that chicks moved closer to the speaker, if they played hen calls, compared to white noise, or alarm calls. They proposed two reasons for why playing hen sounds may improve the feeding rate of chickens: <sup>1)</sup> Hearing the sound may relax the chickens, and <sup>2)</sup> the hen sounds may have decreased the exploratory behaviour of the chickens (Woodcock and Latour, 2004). If this is true, playing hen sounds could make the chickens more relaxed and getting accustomed to their surroundings more easily, and make them more assured that the location is safe. Another issue is the learning effects of hen calls. Auditory signals will serve as localization aids and as an “arousal enhancer” (Fisher 1972) as these auditory signals might mediate the release of noradrenaline (Rickard et.al, 2007). Noradrenaline is critical to the effect of arousal and long term memory (Gibbs and Summers, 2002).



### **3.5 Hypothesis tested in this experiment**

In this experiment I want to examine the relationship between the feeding behaviour of domestic chicks and how hen calls and different prey colouration will affect the amount of prey eaten. I trained the chicks to find and eat mealworms, both alive and dead, before I used them in the experiment, where half of the chicks was presented prey covered with brown neutral colour, and a neutral green covering, whereas the other half was presented prey covered with brown control, and aposematic yellow colour.

My hypothesis was that the chicks would eat most brown prey, fewer bad tasting, and even fewer aposematic bad tasting. Furthermore, my hypothesis was that the chicks that were given sound during eating would eat more prey, compared to chicks not given sound during eating.

## 4 Material and methods

### 4.1. Study subjects

As predators I used a total of 96 ROSS 208 domestic chicks of both sexes in this experiment. They were 1 day old at delivery, from a commercial hatchery, and arrived in batches of 24-26. The chicks were placed in an aluminium cage (60x102x36 cm). The floor was covered with sawdust, with a water station, a feeding station with brown-coloured chick crumbs, and a 250 W heating lamp hanging from the ceiling. The lamp gave a temperature of about 34 C<sup>0</sup>, but for three of the weeks the temperature was so low in the room, that an extra heating oven was placed in the room. Water and food was available *ad lib*, also during the tests. I stayed with the chicks 1 hour after arrival, for socialisation and colour coding them on the head and/or wing(s).

As prey I used mealworms (*Tenebrio molitor*), who were kept alive in fridge temperature, until preparation for tests. They were killed by immersing them in boiling water which killed them instantaneously. The taste manipulated prey was soaked in a solution of 100 ml water, 4% quinine hydrochloride and 2 g mustard powder for 10 minutes. Control prey were soaked in water for the same amount of time.

### 4.2. The training sessions

Each training session was conducted with all 24-26 chicks, where the 16 most eager ones were used in the tests. Both the training and the test trials were conducted in a separate room from where the chicks were kept. A cardboard box was used for both training and test trials, with a rectangle hole with 4 wells, and a sliding lid (see fig 1). Half of the chicks were given sound stimuli (hen cluck) during training, and the other half were not. I recorded the feeding behaviour of the chickens, both in the training and the test, dividing the chickens into 2 training groups and 8 test groups (see table 1). 6 test weeks were conducted, each lasting 6 days, from Monday to Saturday.

The training of the chicks were done in day one and two (Tuesday and Wednesday) of the trial. The training was divided into 8 training sessions, where in the first 4 the chickens were trained in pairs, whereas in the last 4 training sessions they were trained individually,

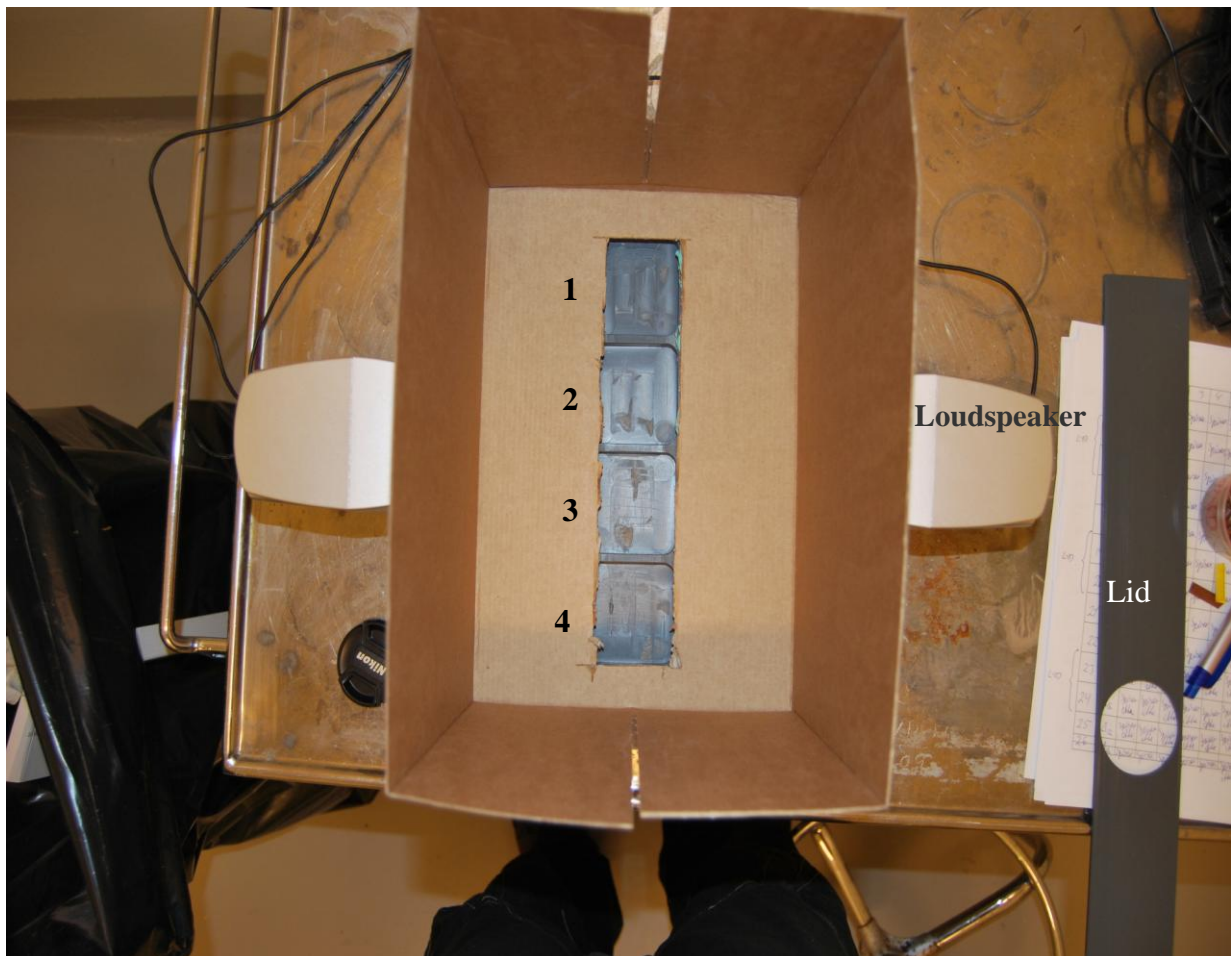
according to table 1. If the chicks started pecking and eating the mealworms, I waited 10 seconds before I removed them from the box. If the chickens did not show any feeding behaviour, I would wait 2 minutes before I removed them from the box, giving them time to adjust to the box.

**Table 1: The training sessions in day 1 and 2**

Training sessions	Description	Day
<b>1</b>	Live mealworms ( <i>Tenebrio molitor</i> ), with no covering, scattered in the bottom of the box. Chickens in pair	<b>1</b>
<b>2</b>	Live and dead mealworms only in wells, with no covering. Chickens in pair	<b>1</b>
<b>3</b>	One live and one dead mealworm in each well, no covering. Chickens in pair.	<b>1</b>
<b>4</b>	One live and one dead mealworm in each well, no covering. One well exposed at a time. Chickens in pair.	<b>1</b>
<b>5</b>	Two dead mealworms in each well, no covering. One well exposed at a time. Chickens individually.	<b>1</b>
<b>6</b>	One dead mealworm in each well, covered with a brown piece of paper shaped as a v, with 4-5 mm of the mealworm visible. Chickens individually.	<b>1</b>
<b>7</b>	One dead mealworm in each well. Two rounds, where the mealworms were covered with 1-2 mm of it visible in round one, and completely covered with a brown piece of paper shaped as a v, in round two. One well exposed at a time. The chickens were placed in a second box between the rounds. Chickens individually.	<b>1</b>
<b>8</b>	One dead mealworm in each well. Two rounds, where the mealworms were covered with 1-2 mm of it visible in round one, and completely covered with a brown piece of paper shaped as a v, in round two. One well exposed at a time. The chickens were placed in a second box between the rounds. Chickens individually.	<b>2</b>

In training sessions 6, 7 and 8 the mealworms were covered with a brown piece of paper. During the training sessions the feeding behaviour of the chickens were noted, to see which chickens were the most interested in the mealworms, based on if they were eating or not and also how eager the chickens pecked at the mealworms. After the first 6 trials were done, I picked out 16 chickens who undertook the two last sessions. If they continued to eat mealworms, I would use these in the test, and if they stopped eating, I would replace these chickens with one of the 9 remaining chickens, taken out after session 6, so that I had 16

chickens after session 8 who pecked and ate mealworms in all 8 training sessions, and therefore would be used in the tests.



**Fig 1:** When the lid was used in the training and test sessions, the wells were exposed one at a time in the order 1, 2, 3 and 4. Loudspeakers on both side of the box were used to create hen sounds.

#### **4.3. The test sessions**

The test sessions were carried out similar to training session 8. The difference here was that I recorded if the chicken ate, pecked or did not show interest in the mealworm for each of the 8 mealworms in the two rounds in each test session. In the test sessions the chickens were placed in the box, and I exposed the wells in order from 1-4. If the chicken did not peck, eat or approach the mealworm within 5 seconds, I would expose the next well, and record the

feeding behaviour of the chicken. The 5 seconds started counting from the time the chicken got aware of the mealworm. It was recorded if the chickens ate, pecked or did not eat the mealworms in the test sessions. The palatable mealworms were in wells 1 and 3, and the unpalatable mealworms were in wells 2 and 4 (see picture 1). The palatable mealworms were covered with a brown piece of paper, and the unpalatable mealworms were covered with either a green or a yellow piece of paper.

**Table 2: The distribution of chicks in different categories. The number of chicks is in parenthesis.**

Training sessions	Sound (8)				No sound (8)			
Test sessions	Sound (4)		No sound (4)		Sound		No sound	
Test sessions	Yellow (2)	Green (2)	Yellow (2)	Green (2)	Yellow (2)	Green (2)	Yellow (2)	Green (2)

In the test sessions the chickens who were given a sound stimuli were given this for the duration of the test session, e.g. sound for both brown and coloured mealworms. The sound was turned on after the chicken was placed in the test box, and was turned off between the two test rounds off each test sessions.

## 4.4 Statistics

I tested for neophobia by using the amount of mealworms eaten in the first test. To compare green to yellow, green to brown and yellow to brown I used fixed effects ANOVA to examine the significance for sound, colour and both sound and colour. To analyze within each sound group I used an independent samples t-test to examine the difference between each of the two colours and brown, and also between green and yellow. I also used the number of brown prey eaten in the first test, and compared this to the number of coloured prey eaten for each treatment group. A value was calculated by subtracting the amount of coloured prey eaten from the amount of brown prey eaten, and I used a single factor ANOVA to examine the

difference between green and yellow. The four sound groups were tested against each other with a single factor ANOVA.

For the average of the first 12 tests, I used an independent samples t-test to compare green and yellow within the same sound group. Independent samples t-test were also used to examine the difference between brown and coloured within the same sound group and colour. Single factor ANOVA was used to examine the difference between the four sound groups, regardless of colour.

When examining the difference between day 1 and day 3, I calculated an average amount of prey eaten in day 1 and an average amount of prey eaten in day 3. Values from day 3 were subtracted from the values from day 1. To compare green to yellow, green to brown and yellow to brown I used fixed effects ANOVA, to examine the significance for sound, colour and the interaction between sound and colour. Independent samples t-test was used to examine between green and yellow, within the same sound group. Independent samples t-test was used to examine the difference between brown and coloured with regards to the difference between day 1 and day 3.

I tested the chick's extinction learning by subtracting the amount of prey eaten in test 13 from the amount of prey eaten in test 15. To compare green to yellow, green to brown and yellow to brown I used fixed effects ANOVA, to examine the effects of sound, colour and the interaction between sound and colour. To examine within each sound group I used an independent samples t-test, to examine the difference between each of the two colours and brown. I also used the difference in the amount of brown prey eaten between test 13 and 15, as for the colours, and compared these with the values from colours, using an independent samples t-test.

## 5 Results

### 5.1 Overall effect

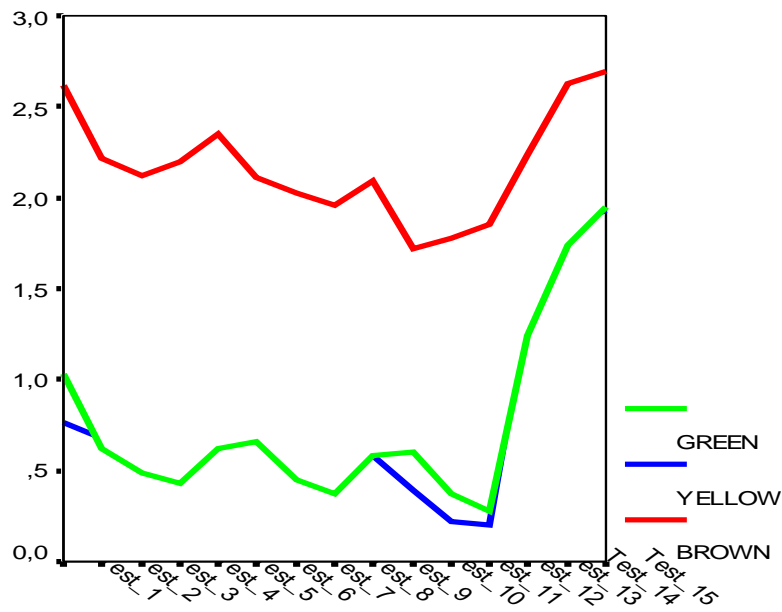


Figure 2: Mean prey eaten of brown, green and yellow during the experiment.

There is a slight difference between green and yellow. In day 1 and 2, the chicks ate slightly more yellow prey, compared to green, and in the 3 day, the average amount of green prey eaten is slightly higher, compared to yellow. Yellow prey is attacked at almost the same rate in day 1 and 2, whilst the green prey shows a steady low decline. The chicks ate considerably more brown prey, compared to both green and yellow (T-test. Df=11,  $p < 0.05$ ) and for the brown too there is a steady decline from day 1 to day 3. The decline rate is actually higher for brown than for green. The chicks discriminate between both brown and green and brown and yellow, but there seems to be little discrimination between green and yellow.

## 5.2 Neophobia in chicks

### 5.2.a Neophobia: Green and yellow

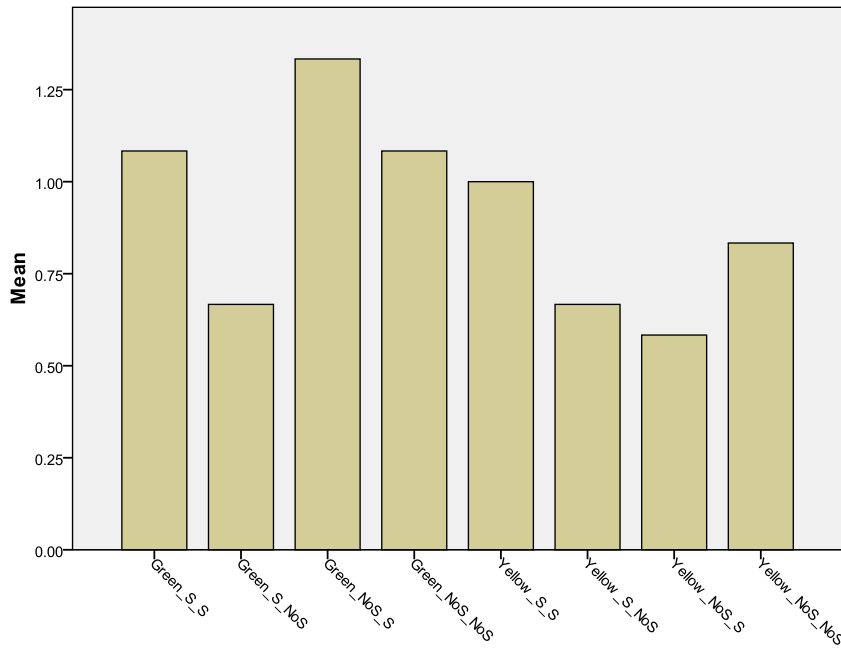


Figure 2: The amount of prey eaten in test trial 1. The general trend is that the chicks given yellow prey show a higher degree of neophobia, compared to chicks given green prey. N for each group is 16.

The fixed effects ANOVA shows no significant difference between green and yellow, when controlling for sound ( $DF=3$ ,  $F=0.711$ ,  $p=0.548$ ) or colour ( $Df=1$ ,  $F=1.926$ ,  $p=0.169$ ). There was no significant interaction between sound and colour either ( $Df=3$ ,  $F=0.741$ ,  $p=0.530$ ).

In the group given sound in training and no sound in test, there is almost no difference between green and yellow. For the other groups, the green prey is attacked more often, compared to yellow prey, but there is no significant difference between green and yellow in any of the sound groups, (T-test.  $Df=11$ ,  $p>0.294$ ). This means that for neophobia, colour has no significant impact on the chicks' aversion.

There is no significant difference between the 4 sound groups ( $Df=3$ ,  $F=0.56$ ,  $p=0.639$ ). This means that in neophobia, sound has no significant impact on the chick's discrimination learning between green and yellow.



## 5.2.b Neophobia: Brown and coloured

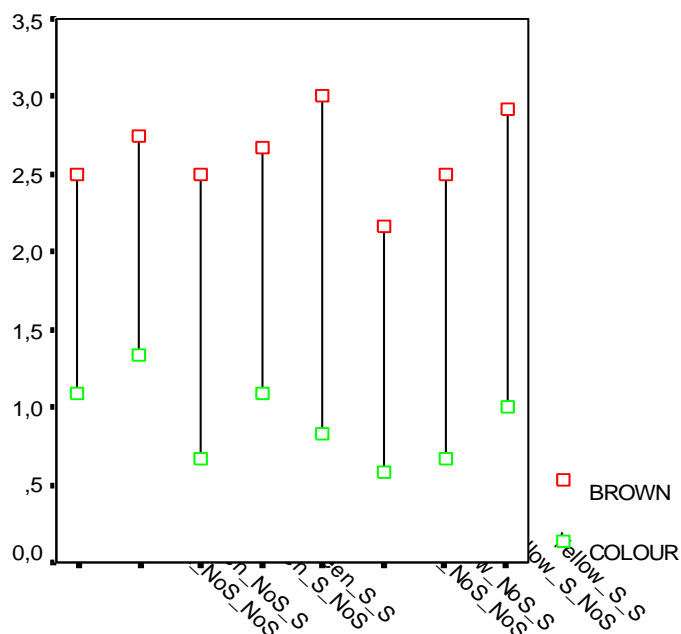


Figure 3 shows the difference between the amount of brown prey eaten, and the amount of green or yellow prey eaten in the 4 green and the 4 yellow groups. In all groups the amount of prey eaten is higher for brown prey, compared to both green and yellow prey, indicating that the neophobia is stronger in all chicks towards conspicuous prey, compared to non-conspicuous brown prey.

The fixed effects ANOVA shows that sound has a significant impact both between brown and green ( $Df=1$ ,  $F=43.6$ ,  $p<<0.0001$ ), and between brown and yellow ( $Df=1$ ,  $F=67$ ,  $p<<0.0001$ ), meaning that sound is important for the chicks discrimination learning between brown and coloured. Colour has no significant impact, and there is no significant interaction between sound and colour.

The amount of brown prey eaten is higher than green and yellow in all groups, thus showing that the chicks have an innate aversion to both green and yellow. There are significant differences between green or yellow and brown in all groups (T-test.  $Df=11$ ,  $p<0.05$ , for all groups). The difference is higher between yellow and brown, compared to the difference between green and brown, with the exception for the chicks given sound in training and no sound in test, where the difference seems to be quite similar. So the chicks given yellow prey shows a higher aversion to the coloured prey than the brown prey, compared to chicks given green and brown prey. The amount of brown prey eaten is slightly higher in the yellow

groups, but there is no significant difference in the amount of brown prey eaten between yellow and green groups (T-test. Df=11,  $p>0.112$ ).

## 5.3 Learning trials

### 5.3.1.a An average of the first 12 trials: Green and yellow

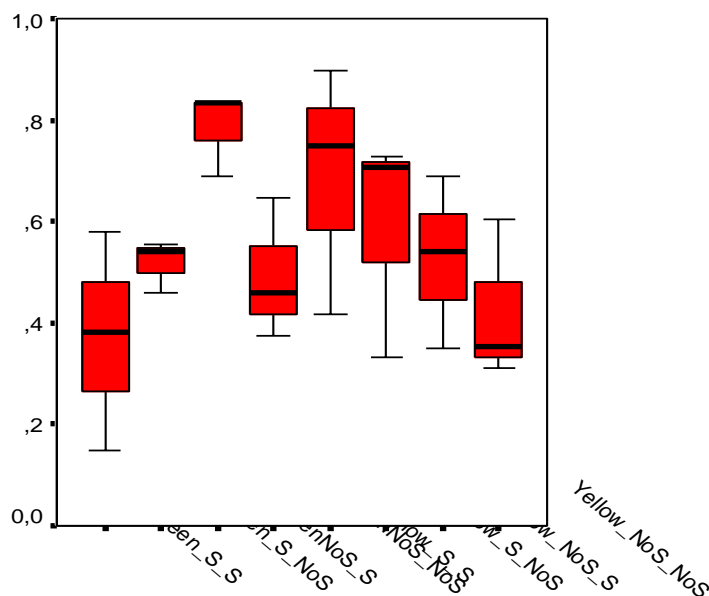


Figure 4 shows the amount of prey eaten in the first 12 trials. For chicks given sound in both training and test, and for chicks given sound in training and not in test, the amount of yellow prey eaten is highest. For chicks given no sound in training and sound in test, and for chicks given no sound in both training and test, the amount of green prey eaten is highest.

For chicks given sound in both training and test there is a significant difference between green and yellow prey eaten in the first 12 tests (T-test. Df=23,  $p=0.03$ ). For the three other groups, there was no significant difference between green and yellow prey eaten in the first 12 tests (T-test. Df=23,  $p>0.20$ ). This shows that sound can have an effect on the chicks feeding rate, although only if they hear the sound from the start (training). For all groups, except chicks given no sound in training and sound in test, chicks given yellow prey, eat more than chicks given green prey, the opposite of what I anticipated. Chicks given green prey and had no sound in training and sound in test have the highest attack rate of all groups, and show also a

small amount of variation, as with chicks given sound in training and no sound in test. Chicks given no sound in training and test show little difference in attack rate over the first 12 tests. There is no significant difference between the 4 sound groups, when I controlled between green and yellow ( $Df=3$ ,  $F=0.56$ ,  $p=0.64$ ), green and brown ( $Df=3$ ,  $F=1.008$ ,  $p=0.39$ ) and yellow and brown ( $Df=3$ ,  $F=0.66$ ,  $p=0.58$ )

### 5.3.1.b An average of the first 12 trials: Brown and coloured

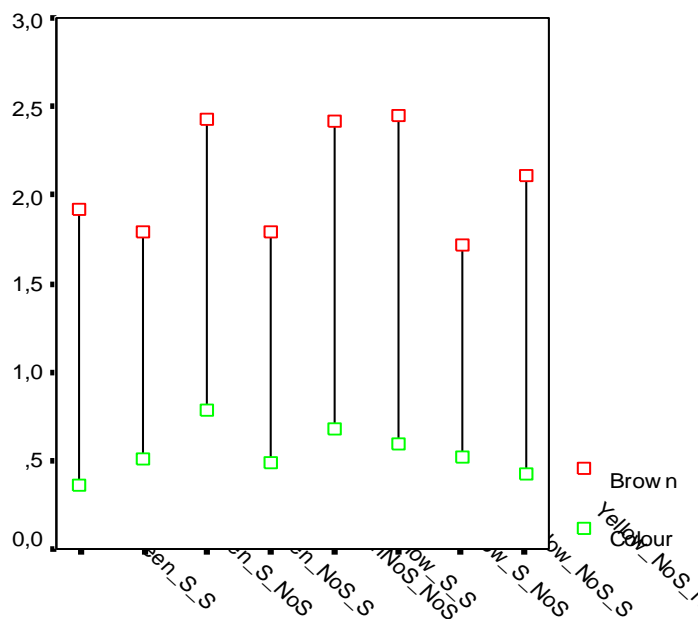


Figure 5 shows the difference between brown and green/yellow (colour) in the average amount of prey eaten in the first 12 tests.

The chicks ate significantly more brown prey compared to green or yellow in all groups (T-test.  $Df=23$ ,  $p<0,05$ ). This will mean that the chicks will learn to discriminate between brown palatable prey and green and yellow unpalatable prey. The amount of difference is higher with chicks given yellow and brown prey, and also these chicks ate more brown prey than chicks given green and brown prey. This shows that the discrimination effect is higher for chicks given yellow and brown prey.

### 5.3.2.a The difference between day 1 and day 3: Green and Yellow

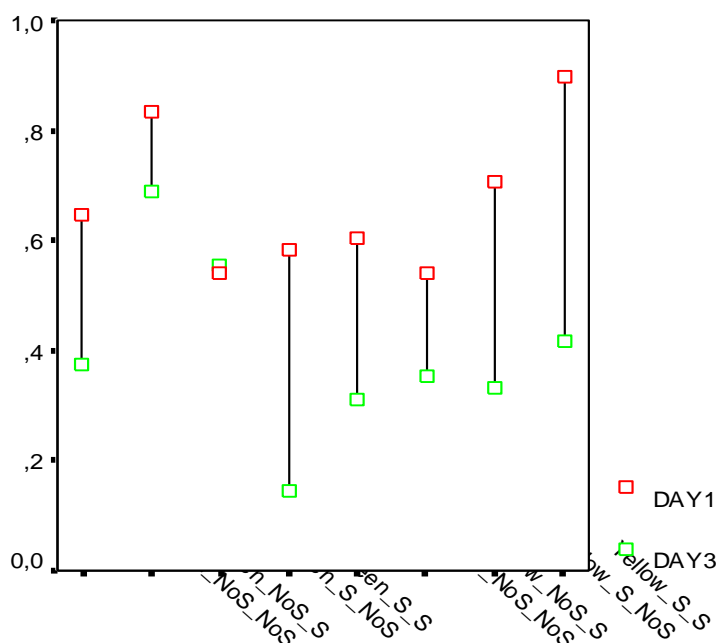


Figure 6 shows the difference in average prey eaten between day 1 and day 3. Chickens given sound in both training and test learned the most, while chickens given sound in training and no sound in test, with green prey, ate more green prey in day 3.

The fixed effects ANOVA shows no significant impact for sound (T-test. Df=1,  $p=0.336$ ) or colour (Df=3,  $p=0.347$ ), and no significant interaction between sound and colour (Df=3,  $p=0.694$ ).

For chicks given green prey, there is a tendency for significant difference between the amount of prey eaten in day 1 and day 3 for chicks given sound in both training and test (T-test. Df=11,  $p=0.071$ ), whilst there is no significant difference in the three other groups given green prey (T-test. Df=11,  $p>0.20$ ).

For chicks given yellow prey there is significant difference between day 1 and day 3 for chicks given sound in both training and test (T-test. Df=11,  $p=0.038$ ) and a tendency for significance for chicks given sound in training and no sound in test (T-test. Df=11,  $p=0.068$ ). For the two other groups of chicks given yellow prey, there was no significant difference between the amount of yellow prey eaten in day 1 and day 3 (T-test. Df=11,  $p>0.16$ ). There is

not significant difference on the difference between day 1 and day 3 between green and yellow, within the same sound group (T-test. Df=23,  $p>0.17$ ), except for in chicks given sound in training and no sound in test (T-test. Df=23,  $p<0.05$ ). There is no significant difference between the 4 sound groups (Df=3,  $F=1.13$ ,  $p=0.33$ ).

### 5.2.2.b The difference between day 1 and day 3: Brown and coloured

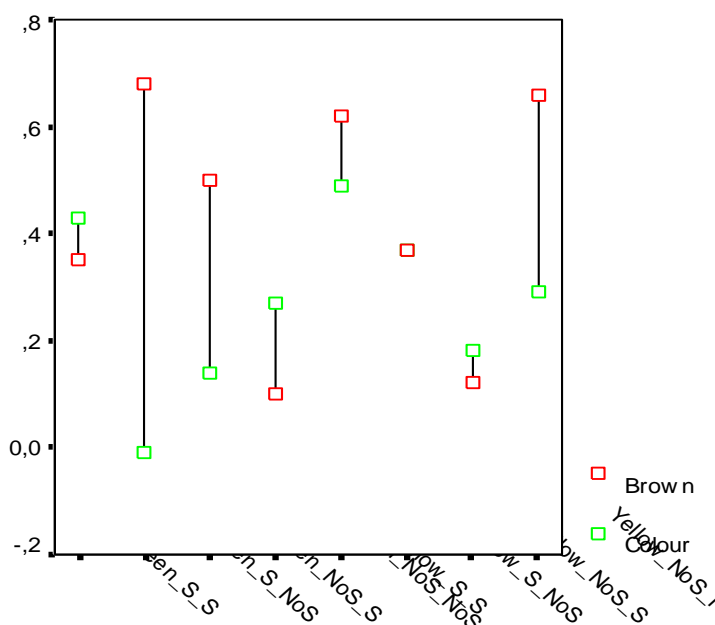


Figure 7 shows the difference between brown and green/yellow prey. The amount of brown and colour is calculated as the mean difference between day 1 and day 3. For chicks given sound in training and not the test, there is a large difference between green and brown, but there is no difference between brown and yellow.

The fixed effects ANOVA shows that sound has a significant impact both between brown and green (Df=,  $F=66.17$ ,  $p<<0.05$ ) and between brown and yellow (Df=,  $F=66.9$ ,  $p<<0.05$ ). Colour had no significant impact (Df=1,  $p>0.376$ ) and there was no significant interaction between sound and colour, in neither green nor yellow (Df=3,  $p>0.587$ ).

The difference between brown and green, with regards to the difference between day 1 and day 3 shows no significant difference in any of the 4 green groups (T-test. Df=1,  $p>0.28$ ).

This means that the decline in prey attacked is not significantly different for green compared to brown.

There is no significant difference between brown and yellow either, with regards to the difference between day 1 and day 3 for brown and yellow ( $Df=1$ ,  $p>0.36$ ). Also here the decline in prey attacked is not significantly different for yellow compared to brown.

The highest difference is for chicks given sound in training and no sound in test, whereas for chicks given yellow there is no difference at all.

## 5.4 Extinction learning

### 5.4.a Extinction learning: Green and yellow

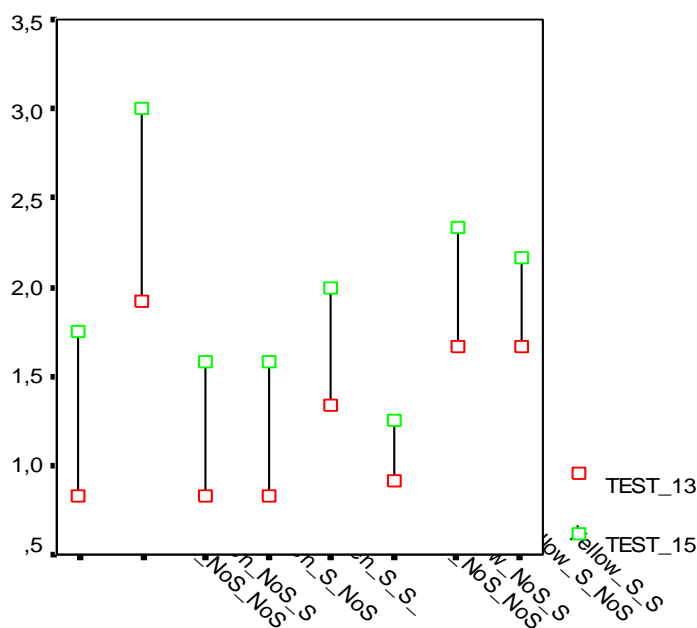


Figure 8 shows the difference between test 13 and test 15 in the amount of green and yellow prey eaten. The difference is larger for chicks given green prey, compared to chicks given yellow prey, indicating that the yellow colour sticks more to the chick's memory.

In all groups there is an increase in number of prey attacked from test 13 to test 15 (extinction tests). Chicks given green prey show an overall slightly higher increase in prey attacked.

There is a weak significant difference between test 13 and 15 for chicks given green and no sound in both training and test (T-test. Df=11,  $p=0.059$ ) and for chicks given no sound in training and sound in test (T-test. Df=11,  $p=0.059$ ). For the two other green groups there is no significant difference between test 13 and test 15 (T-test. Df=11,  $p>0.132$ ).

There is no significant difference between test 13 and 15 for chicks given yellow prey (Df=11,  $p>0.281$ ). This will also mean that the chicks have a stronger memory towards yellow prey, and its unprofitability, compared to chicks given green prey.

There is no significant difference between green and yellow, within the same sound group, when I used the difference between test 13 and 15 (T-test. Df=23,  $p>0.259$ ). This mean that the increase in prey attacked from test 13 to 15 is not significantly higher for green than it is for yellow. There is no significant difference between the 4 sound groups (Df=3,  $F=0.057$ ,  $p=0.981$ ).

#### 5.4.b Extinction learning: Brown and coloured

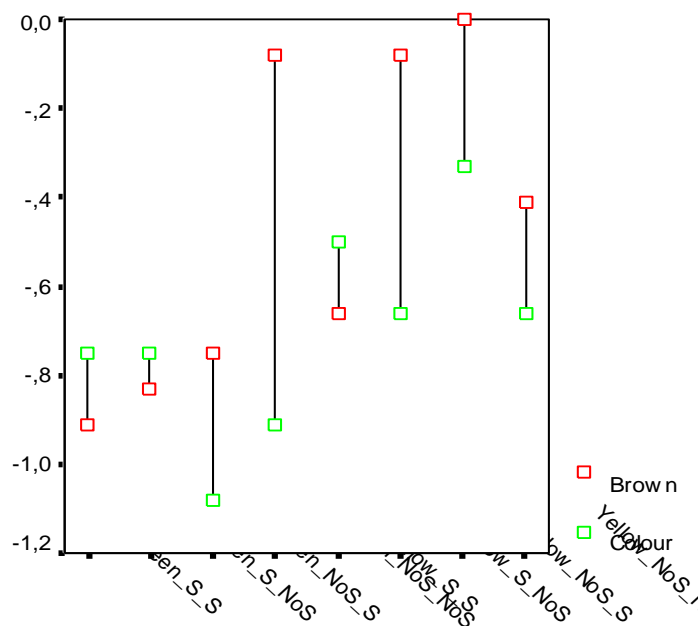


Figure 9 shows the difference between brown and green/yellow (colour) prey. The value is calculated as the difference between test 13 and test 15 for brown, green and yellow.

There is no significant difference between brown and green, with regards to the difference between test 13 and test 15 (T-test.  $Df=11$ ,  $p>0.082$ ). The difference between green and brown that were given no sound in both training and test has tendencies to significant (T-test.  $Df=11$ ,  $p=0.082$ ). There is also no difference between brown and yellow, with regards to the difference between test 13 and 15 (T-test.  $Df=11$ ,  $p>0.137$ ). This means that there is no significant difference in the increase in prey attack between green and brown, or between yellow and brown.



## 6. Discussion

### 6.1 Neophobia

In the first test there was no significant difference between the amounts of green or yellow prey attacked, although there is less yellow prey being attacked, compared to green. I was expecting a difference here, since yellow is an aposematic colour (Rowe and Guilford, 1999). The innate aversion towards yellow colouration means that the chicks should respond negative towards yellow, and more positive to green, even though the difference here is small. There is however significant differences between both brown and green and brown and yellow, which shows that the chicks discriminate already in the first test.

I often noted that chicks were eager to find food under the brown piece of paper, but was not specially eager to look for food under the green or yellow paper, and this shows that the chicks shows neophobia towards both yellow and green colours. Johnson (2007) also notes the same results, indicating neophobia in chicks. Johnson (2007) notes that another explanation for this outcome is because the chicks could have problems finding the prey under the paper hats, and this indicates that this way of colouring the prey might not be optimal for the experiment output. An alternative could be to colour the prey, as both Mappes and Altalos (1997) and Rowe and Guilford (1999a) has done before. In my experiment, however, I noted that the chicks pecked at the coloured paper hats, but often lost interest, and I will assume it's due to neophobia, but it would be interesting to see if colouring the prey would yield a different outcome. I used 8 training trials, and the question is if this was enough time for the chicks to learn. However, most of the chicks ate all the prey on the last training session, and the test started the same day, so I will anticipate that the learning effect is still strong with the chicks, in comparison to the learning effect from day 1 to day 2 in training. The chicks ate less prey on the first training on day 2, than on the last training on day 1. Sound had no significant effect on the attack rate between green and yellow, but had a significant effect between brown and coloured. Since the hen sound was played both when the chicks were shown the brown and the coloured prey, the sound could make the chicks more relaxed, and making the chicks explore less, and eat more brown prey (Woodcock and Latour, 2004), and the reduced prey search under green and yellow papers, could explain the lack of green and yellow prey being attacked.

## **6.2 The Learning period**

### **6.2.a The overall effect**

The overall effect is that both coloured prey and brown prey is eaten less from test 1 to test 12. I was expecting the amount of brown prey eaten from test 1 to test 12 to be fairly constant, since the chicks grew in size during this time, thereby demanding more food on day 3, compared to day 1. I noted that the chicks that pecked at the coloured food, but did not eat it, often did not show any interest in the brown prey. The chicks would eat the first brown prey, but not the second, if it had pecked on the first coloured. When the chick pecked at the coloured prey, the taste agent could leave residue on the chicks beak, and the question is if this could influence what the chicks smell, and thereby influence the chicks choice not to peck at the second brown. This could explain the decline on the brown from day 1 to day 3, being higher than that of the green and yellow, because of the chicks response to brown prey after encountering a coloured prey.

If the chicks had been given more than 5 seconds to respond to the prey, the result might be different, something that could be interesting to do further study on.

Johnson (2007) had a small amount of yellow prey being attacked, and states that the innate fear of yellow may have overshadowed the positive effect of the hen sounds. In my results on the other hand, the chicks given yellow prey, eats more than chicks given green prey in the first two days. The difference is however small, and could be due to variation within chicks given green and yellow prey.

### **6.2.b Learning tests**

The results were quite puzzling, because I was expecting the average of green prey attacked to be higher than yellow. The fact that chicks given yellow prey ate more in the presence of hen sound, shows how sound might be a factor that will contribute to the feeding behaviour of chicks. Chicks given green prey also show a high attack rate in the group given no sound in training and sound in test, which also indicates that sound plays a role in chicks feeding behaviour. As I noted during the tests, chicks given sound in test showed a more relaxed behaviour, and tended to stay put the first seconds, in comparison to chicks given no sound,

which tended to start moving about at once. The chicks given sound could be using these first seconds to try to locate the sound, and maybe try to see what the mother hen is trying to show them. Rowe (2001) states that in the presence of a tone the chicks will learn to discriminate faster, and this is true if one look at the difference between colour and brown.

For chicks given yellow prey, hen sound will have a positive effect on the attack rate, but when given green prey, sound only have a positive effect when the chicks are not accustomed to it. This could again be that the chicks given sound in test are more relaxed and therefore will eat more, but this doesn't explain why chicks given green and sound in both training and test eats the second least amount of prey. Between coloured prey and brown there is significant difference, and this can show that the colour have a significant impact on prey attack. This difference is however only between brown and colour, and again, this could also be explained by the experimental design, as explained above.

Although there was no significant difference between the 4 sound groups, one can not say that sound is not important. The reason for this is that there are significant differences between the green and yellow, when given sound in both training and test. This would mean that the combination of sound and colour is significant in this case. There are not significant differences in the other groups, and the non-significant difference in the other group given sound in test, undermines this result.

There was no significant impact on sound, colour or a combination of these in the difference between day 1 and day 3, which I find a bit strange. For both green and yellow there is a significant difference between day 1 and day 3 when given sound in both training and test, which I think only states that sound do have an impact on the chicks memory and learning. However, again, the fact that there is no significant difference for the other groups given sound in test undermines this result. And the fact that chicks given yellow prey and sound in training and no sound in test also show a significant difference doesn't help. The fact that there is no significant difference between the 4 sound groups also enhances the non-significant importance of sound in this experiment.

Since the chicks have a similar decline in prey attack for brown, green and yellow, shows that the colour of the prey are not important in the learning effect for these chicks, as they did not get any better at discriminating between the colours and brown.

### 6.3 Extinction learning

The aversion towards green and yellow decreases rapidly from test 13 to test 15. The chicks do not eat as many green or yellow, as they do brown, but the increase is higher for coloured prey. This shows that the chicks will rapidly start to eat palatable food again. A possible explanation for the difference in increase between coloured and brown prey, could be because the chicks ate a small amount of coloured prey during the learning tests, whereas brown prey was eaten at a high rate during the learning tests. Two possible reasons for why the chicks eat less coloured prey in test 15, compared to brown, could be due to fear of bad tasting prey, or dietary conservatism. Dietary conservatism is when an animal refuses to eat fully palatable food (Marples. et.al. 1998) even though it might be some time since it last encountered an unpalatable food item of same sort. Dietary conservatism will make the animal refuse to extend the diet, and not based upon fear of the aposematic coloured food (Marples et.al, 2007), and this was true for some chicks, but overall, the chicks showed that they included coloured prey into their diet, thereby showing little signs of dietary conservatism.

Johnson (2007) had the problem with very few chicks trying to eat yellow prey on the 4<sup>th</sup> day, and only starting at the last tests, whereas in my experiment, the chicks show a large increase from test 12 and 13, with the a smaller incline up to test 15. This result show that the chicks would try to peck at the coloured food, even though they knew it would be distasteful. The chicks would peck at the prey in the tests in the learning period, but in the latest learning tests, the pecking subsided, as many of the chicks learned to discriminate between brown and coloured. So the question would be why the chicks started pecking in test 13 again. A plausible explanation could be that the chicks had forgotten some of what they learned in test 12, before starting at test 13. The same is seen between the separate days, where the chicks eat more prey first test the next day, compared to the last test the previous day.

Another possible explanation could be that the chicks could smell the taste agent during the learning tests, and this would be a contributing factor in their attack rate, and that the lack of smell in test 13-15 could influence the chicks to try pecking at the prey. Marples and Roper (1995) found that odours naturally associated with chemical defences in insects and plants enhance neophobia in chicks, but only when presented with a novel coloured prey.

Nevertheless, this states that smell could influence the chicks response.

## 7 Conclusion

There was statistically difference between coloured and brown, but it was not confirmed a statistically difference between the neutral green and aposematic yellow, and the trend also pointed in the opposite direction when seeing the learning tests as a whole. But yellow show a higher degree of learning from day 1 to day 3. Sound did seem to have an impact on the chicks attack rate, but this was not confirmed statistically. In the first test sound had a positive impact on the chicks feeding rate, but during the whole learning tests, sound also had a positive effect on the learning ability.

In the extinction tests, sound showed little effect on the chick's food search, as the difference between the groups is minimal. Chicks given green prey show a higher degree of extinction learning, even though it is not confirmed statistically.

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